Wine – Making using Freeze Dried Immobilized Cells into a Catalytic Multistage Fixed Bed Tower (MFBT) Bioreactor

V. Sipsas[1*], G. Kolokythas[1], M. Kanellaki[1], B. Bugarski[2] and V. Nedovic[3]
[1]Food Biotechnology Group, Section of Analytical Environmental and Applied Chemistry, Department of Chemistry, University of Patras, GR-26500 Patras, Greece (Contact e-mail: bill_sips@hotmail.com); [2]Dept. of Chemical Engineering, Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia; [3]Dept. of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Belgrade, Serbia

Intoduction

Over the last few decades there has been an upsurge of interest in immobilized cells due to attractive technical and economic advantages compared to the conventional free cell system (Margaritis, 1994; Nedovic and Willaert, 2005). In the scope of yeast immobilization one of the developed procedures is the immobilization of psychrophilic and alcoholtolerant yeast strains on gluten pellets and freeze drying of the immobilized culture. These dried immobilized biocatalysts have been used for winemaking and brewing leading to final products with improved quality and organoleptic character (Bekatorou, 2001; Bekatorou, 2002; Iconomopoulou, 2000; Iconomopoulou, 2002; Iconomopoulou, 2003). Recently, a catalytic Multistage Fixed Bed Tower (MFBT) bioreactor was proposed for potable alcohol production from molasses in industrial scale resulting to higher alcohol production rate (Bakoyianis, 1996; Koutinas, 1997). The aim of the present study, hence, was to investigate batch wine production in a vertical MFBT bioreactor by freeze dried immobilized yeast cells and to compare this bioreactor system to a packed bed bioreactor in regards of fermentation kinetics and wine quality.

Materials and Methods

AXAZ-1, an alcohol resistant and psychrofilic *Saccharomyces cerevisiae* yeast strain isolated (Argiriou, 1992) from the Greek agricultural area, was used in the present study. Yeast cells were immobilized on gluten pellets (GP), on delignified cellulosic materials (DCM) and on apple pieces (AP). Immobilization process was carried out as described previously (Bardi, 1996; Bardi, 1994; Kourkoutas, 2001). For technical reasons only gluten supported biocatalyst was frozen to -45^{o}C with a cooling rate of 3^{o}C/min and without use of protecting media. The frozen samples were freeze-dried overnight at 5.10^{-3} bar and at -45^{o}C in a Freeze Dry System, Freezone 4.5 (Labconco) (Iconomopoulou, 2002). The above freeze dried biocatalyst was used for repeated fermentation batches of grape must, that were carried out separately into (a) a catalytic Multistage Fixed Bed Tower (MFBT) bioreactor and (b) a packed bed bioreactor (PBB). Specifically, it was studied the effect of different fermentation temperatures on alcoholic fermentation kinetic parameters and on the production of volatile by products. At the end of every batch in both PBB and MFBT bioreactors, samples were collected and analyzed for ethanol (using Gas Chromatography), residual sugar (using High Performance Liquid Chromatography), volatile by-products (using Gas Chromatography), total (titration) and volatile (titration after distillation) acidity and free cells concentration (using Spectrophotometric methods).

Results and Discussion

Immobilization of yeast cells on GP has been confirmed by Scanning Electron Microscopy (SEM) (Figure 1), as well as by the repeated batch fermentations and by the clarity of the fermented liquid. The

very low concentrations of free cells (0.4-2.2 and 0.6-2.1 g/L in MFBT bioreactor and PBB bioreactor, respectively) indicate that fermentations were carried out by the freeze-dried immobilized cells.

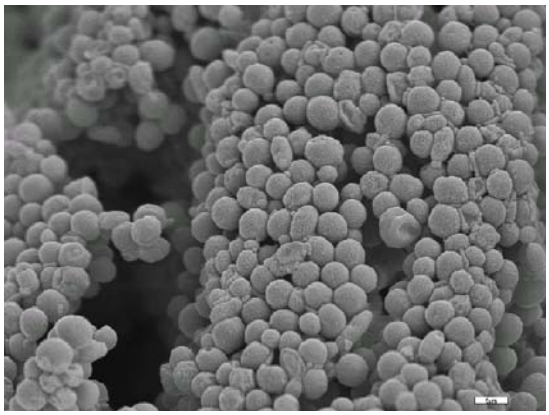

Figure 1. Freeze dried immobilized AXZ-1 yeast cells on gluten pellets.

Freeze-drying of immobilized AXAZ-1 cells on gluten pellets was carried out at a cooling rate of 3^{o}C/min without using any protecting medium, providing an easy to handle and cost-effective method, reducing the contamination risk of the final product with a residue from the protecting media that could affect its quality characteristics.

Then, freeze dried immobilized cells were added in the fermentation broth and the fermentations proceeded rapidly in both cases, after an adaptation time of about 3 h. Both systems showed an important operational stability and no decrease in activity, even at low temperatures (5^{o}C). The use of MFBT bioreactor compared to the use of PBB bioreactor and to conventional fermentations led to higher production rates of wine and ethanol and to lower fermentation times. Total and volatile acidities of the produced wines (0.08 – 0.28 g of acetic acid/L and 4.4 – 6.3 g of tartaric acid/L) were similar to those contained in commercial products and total acidity was decreased when fermentation temperature was dropped due to increased crystallization of potassium tartrate as the temperature decreased to lower values. (Kourkoutas, 2001; Kourkoutas, 2002; Bakoyianis, 1992). Additionally, low values of residual sugars, high conversion rates and ethanol concentrations (10.3 – 12.1 % v/v), confirm that freeze-dried immobilized biocatalyst is suitable for dry wine making, at all studied temperatures (Table 1).

As freeze-dried immobilized cells on GP proved to be suitable for wine fermentation in MFBT bioreactor, the final consideration was the evaluation of the fermented products quality. Therefore, concentrations of volatile by-products were monitored during repeated batch fermentations of grape must (Table 2). In the present study, acetaldehyde content up to 76mg/L for MFBT and up to 54mg/L for PBB respectively, were observed, but in most cases they were lower and similar to those found in traditionally produced wines. Concentrations of higher alcohols (1-propanol, isobutyl alcohol and amyl alcohols) were low, as it is obvious in Table 2. The production of amyl alcohols proved to be temperature dependent and decreased at low temperatures. Ethyl acetate concentrations ranged in levels usually detected in wines and increased with temperature drop, but it still remained in acceptable levels (<150ppm) (Jackson, 1994). The reduction of amyl alcohols and increase of ethyl acetate concentrations in decreased temperatures have been related with improvement of organoleptic quality (Etievant, 1991; Vidrih, 1999). Finally, the methanol content ranged <100mg/L in all cases contributing to the improved quality of the final products.

	Fermentation Temperature (^{o}C)	Fermentation Batch	Initial ^{o}Be density	Fermentation time (h)	Wine Production Rate (g/l/day)	Residual Sugar (g/l)	Ethanol (% v/v)	Ethanol Production Rate (g/l/day)	Conversion (%)
MFBT	30	1-5	12	62	484	7.5	12.1	37	96
	20	6-10	12	93	323	5.5	11.9	24	97
	10	11-13	11.4	214	140	9.2	10.7	8.6	95
	5	14-15	11.8	740	41	8	10.9	3	96
PBB	30	1-5	12	62	484	5.5	11.9	36	97
	20	6-10	11.8	105	286	4.2	10.9	20	97
	10	11-13	11.2	240	126	9.8	10.3	7	95
	5	14-15	11.8	812	37	11	10.6	2.5	92

Table 1. Effect of temperature on fermentation kinetic parameters observed in grape must fermentation by freeze dried immobilized yeast cells, on gluten pellets, using a) a MFBT batch bioreactor and b) a packed bed batch bioreactor (PBB).

	Fermentation Temperature (^{o}C)	Fermentation Batch	Acetaldehyde (mg/l)	Ethyl acetate (mg/l)	1-Propanol (mg/l)	Isobutyl alcohol (mg/l)	Amyl alcohols (mg/l)	Methanol (mg/l)
MFBT	30	1-5	76	43	33	70	68	63
	20	6-10	75	35	24	50	71	55
	10	11-13	33	57	23	22	39	83
	5	14-15	12	79	16	11	22	46
PBB	30	1-5	54	29	32	79	92	54
	20	6-10	28	61	23	57	75	51
	10	11-13	57	60	28	27	40	48
	5	14-15	28	67	13	10	31	52

Table 2. Effect of temperature on formation of volatiles in grape must fermentation by freeze dried immobilized yeast cells on gluten pellets, using a) a MFBT batch bioreactor and b) a packed bed batch bioreactor (PBB).

Conclusions

To sum up, freeze-dried immobilized AXAZ-1 yeast cells on GP was proved to be a suitable biocatalyst for batch wine-making in a Multistage Fixed-Bed Tower (MFBT), resulting in improved wine and ethanol productivities compared to packed bed bioreactor (PBB) and to conventional fermentations. The wines produced in both systems at low temperatures were of improved quality as they contained low levels of higher alcohols and increased concentrations of ethyl acetate. The above results can lead to a possible industrial application of the process.

Acknowledgment

This work has been supported by the funds of bilateral project between Greece and Serbia, titled "Wine and beer making by freeze dried immobilized cells in different bioreactor systems", 2005-2007.

References

Argiriou, T. et al. (1992). *New alcohol resistant strains of S. cerevisiae species for potable alcohol production using molasses.* Appl. Biochem. Biotechnol., 36, 153-161.

Nedovic, V. and Willaert, R. (2005) *Applications of Cell Immobilisation Biotechnology*, Springer, Dordrecht/Berlin/Heidelberg/New York, pp. 1-573.

Bakoyianis, V. et al. (1992). *Low-temperature wine-making by immobilized cells on mineral kissiris.* J. Agric. Food Chem., 40 (7), 1293-1296.

Bakoyianis, V. et al. (1996). *A catalytic Multi-Stage Fixed Bed Tower (MFBT) in an Industrial Scale Pilot Plant.* Biotechnol. Bioeng , 49, 197.

Bardi, E. et al. (1994*). Immobilization of yeast on delignified cellulosic material for room temperature and low-temperature wine making*. J. Agric. Food Chem., 42, 221-226.

Bardi, E. et al. (1996). *Room temperature and low temperature wine making using yeast immobilized on gluten pellets.* Process Biochem., 31 (5), 425-430.

Bekatorou, A. et al. (2001). *Low-temperature brewing by freeze-dried immobilized cells on gluten pellets.* J. Agric.Food Chem., 49 (1), 373-377.

Bekatorou, A. et al. (2002). *Low-temperature brewing by freeze-dried immobilized cells.* Appl. Biochem. Biotechnol, 97, 105-121.

Etiévant, P.X. (1991). *Wine. In Volatile Compounds in Foods and Beverages*, Henk Maarse Ed.; The Netherlands, Zeist; pp. 483-546.

Iconomopoulou, M. et al. (2000). *Delignified cellulosic material supported biocatalyst as freeze-dried product in alcoholic fermentation.* J. Agric. Food Chem., 68 (3), 958-961.

Iconomopoulou, M. et al. (2002). *Low temperature and ambient temperature wine making using freeze-dried immobilized cells on gluten pellets.* Process Biochem., 37, 707-717.

Iconomopoulou, M. et al. (2003). *Effect of freeze-dried cells on delignified cellulosic material in low-temperature wine making.* Appl. Biochem. Biotechnol., 104, 23-36.

Jackson ,S.R. (1994). In: *Wine Science. Principles and Application*, Academic Press; pp. 184, 201, 223.

Kourkoutas, Y. et al. (2001). *Wine Production Using Yeast Immobilized on Apple Pieces at Low and Room Temperatures.* J. Agric. Food Chem., 49 (3), 1417-14.

Kourkoutas, Y. et al. (2002). *Continuous wine fermentation using a psychrophilic yeast immobilized on apple cuts at different temperatures.* Food Microbiol., 19, 127-134.

Koutinas, A.A. et al. (1997). *Qualitative Outline to Industrialize Alcohol Production by Catalytic Multi-Stage Fixed Bed Tower (MFBT) Bioreactor.* Appl. Biochem. Biotechol, 66. 121-131.

Margaritis, A. et al. (1984). *Advances in Ethanol Production using Immobilized Cell Systems.* CRC Crit. Rev. Biotech., 1 (4), 339-393.

Vidrih, R. et al. (1999). *Synthesis of higher alcohols during cider processing.*, Food Chem., 67, 287-294.